Effect of IL6 -174 G/C Gene Polymorphism on Response to Interleukin-6 Blocking Therapy in Systemic Juvenile Idiopathic Arthritis

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Abstract: *Objective*: The main objective of this study was to evaluate the association of IL6-174 G/C gene polymorphisms and the response to tocilizumab (TCZ) in patients with systemic juvenile idiopathic arthritis (s-JIA).

Methods: Sixty patients with s-JIA (37 males and 23 females with median age at onset of 5.2 years) who received TCZ were recruited. Basic demographic, laboratory and clinical data were collected alongside the IL-6 haplotype status. The overall response to treatment with TCZ was assessed according to a number of variables including the extent of disease activity reduction, the achievement of clinically inactive disease, the necessity to switch to another biologic disease modifying anti-rheumatic drug (bDMARD) and the achievement of a glucocorticoid-free state.

Results: Three IL6 -174 genotypes, including, GG, GC, and CC were found with higher frequencies of GC genotype. These genotypes had non-significant association with the response of s-JIA patients to IL-6 blockade in this cohort study. However, a longer time frame from disease onset to diagnosis was associated with poorer long-term treatment response.

Conclusion: We observed no significant impact of IL6 -174 G/C gene polymorphisms on treatment response to TCZ in s-JIA Egyptian patients. The observation that a shorted timeframe between symptom onset and diagnosis is associated with better long-term response to TCZ provides evidence for a therapeutic "window of opportunity" in patients with s-JIA.

Keywords: Interleukin 6, Systemic Idiopathic Arthritis, Interleukin-6 blockade therapy, IL6 gene polymorphisms.

1. INTRODUCTION

Juvenile idiopathic arthritis (JIA) is one of the most common causes of chronic childhood disability. Approximately 11% of patients with JIA suffer from the systemic-onset (s-JIA) form that in most cases is characterized by severe, debilitating, extra-articular features and occasionally fatal complications [1, 2]. Children with s-JIA are often exposed to potentially toxic therapies for many years in order to achieve remission or at least a satisfactory disease control. Unfortunately, however, many children still experience early joint destruction, requiring surgical replacement. Moreover, up to 48% of these patients will still have active disease 10 years after the onset [3]. The clinical spectrum of active disease encompasses spiking fever, an evanescent macular rash, lymphadenopathy, hepatosplenomegaly, serositis, myalgia, and arthritis. As far as laboratory abnormalities are concerned, anemia, markedly increased acute phase reactants, fibrinogen, neutrophil and platelet counts are often observed. Patients may also display polyclonal hypergammaglobulinemia and in severe cases raised liver enzymes and a coagulopathy may also occur [4]. Additionally, inflammatory cytokines, such as tumor necrosis factor (TNF)-alpha, IL-1 and IL-6 were found at high level in the serum and synovial fluid of inflamed joints [5-8].

IL-6 has a broad spectrum of action including the ability to induce and maintain survival, proliferation, and differentiation of inflammatory cells in particular T and B lymphocytes. [9,10]. Its pro-inflammatory role is of paramount importance in the pathogenesis of s-JIA since IL-6 can drive various events within innate and adaptive immune response, control leucocyte recruitment and ultimately trigger and maintain the inflammatory infiltrate [11]. In addition, IL-6 can elicit a

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E-ISSN: 2310-9874/22

number of hormonal responses, including control of vascular function, insulin resistance, lipid metabolism and iron transport; it also has an impact on neuro-endocrinal and neuropsychological behaviour [12].

Over the last few decades, therapuetic approaches directly or indirectly targeting soluble mediators such as TNF-alpha, IL-1 and IL6 have been increasingly used for the treatment of rheumatic diseases, including s-JIA [13] . Tocilizumab (TCZ) is a humanized anti–IL-6R monoclonal antibody (IgG1) licensed for use in s-JIA. Although being capable to offer a significant benefit along with a good safety profile in patients with s-JIA [14], still a consistent number of patients respond partially or not at all to TCZ. The heterogenous response to IL-6 blockade may be attributed to a single nucleotide polymorphism (SNP) in IL-6, where haplotype-tagging SNPs (htSNPs) however may explain patients association with s-JIA risk and the response to treatment [15,22,23].

Therefore, the aim of the present study was to evaluate the association of IL6-174 G/C gene polymorphisms with response to the IL-6 blockade in a cohort of patients with s-JIA treated with TCZ.

2. METHODS

2.1. Patient Cohort and Clinical Assessment

We conducted an observational cohort study enrolling patients with s-JIA who were treated with TCZ during the course of the disease in the outpatient Clinic, Department of Physical Medicine, Rheumatology & Rehabilitation, Tanta University Hospitals, Egypt, between June 2020 and January 2021. Patients were diagnosed as having s-JIA if they met the International League of Associations for Rheumatology (ILAR) classification criteria 2001 for this JIA category [16]. Exclusion criteria were a diagnosis of other JIA subset and a diagnosis of other rheumatic diseases (including autoinflammatory diseases). The study was conducted in accordance with the Declaration of Helsinki and approved by the ethical committee, Faculty of Medicine, Tanta University, Egypt. Written informed consent was obtained from children's parents before enrollment. We followed the recommendations of STROBE guidelines during the preparation of this manuscript.

At baseline, we collected demographics data as well as information on disease characteristics and current and previous medications for s-JIA and comorbidities.

The clinical response to TCZ was defined as follows: 1) Good response, if the signs and symptoms of active disease (fever, rash, adenopathy, hepatosplenomegaly, serositis, and arthritis) had resolved and if the levels of inflammation markers (C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) had improved by at least 50% following treatment with TCZ, and if this response was maintained for at least 6 months; 2) Transient response, if there was an initial response according to the parameters of good response for at least 2 months followed by recurrence of disease; 3) Poor response, if the above-mentioned parameters for improvement were not met [17].

The number of patients with poor or transient response who switched to TNF blockers following TCZ was also recorded. This allows to estimate the long-term efficacy of treatment with TCZ.

For secondary analyses, we used the following additional improvement criteria: 1) any response, i.e., improvement of fever (if present) and/or arthritis (if present), defined according to the criteria used by Arthur *et al*.; [18] 2) improvement in the 0-10 physician global assessment (PhGA) by 30%, 50%, 70%, or 90%; 3) improvement in the modified Juvenile Arthritis Disease Activity Score in 10 joints (JADAS-10), consisting of the sum of the PhGA (scale 0–10), the count of joints with active arthritis (scale 0–10), and normalized C reactive protein (CRP) level (scale 0–10; calculated as [CRP (in mg/liter) − 10]/10, with a CRP level of <10 mg/liter representing a score of 0), 4) clinically inactive disease (CID) within 6 months of TCZ treatment, defined according to the CID criteria of a PhGA = 0 a CRP level or erythrocyte sedimentation rate (ESR) within the normal range, and no documentation of active arthritis, fever, rash, adenopathy, hepatosplenomegaly due to s-JIA, uveitis, or morning stiffness, and 5) Achievement of a glucocorticoid-free state within 6 months of TCZ initiation [19,20]

2.2. Genotyping for IL6 – 174 G/C Polymorphism

Genomic DNA was extracted from the whole peripheral blood sample with EDTA using the GeneJET Whole Blood Genomic DNA Purification Mini Kit supplied by (Thermo Scientific, Waltham, Massachusetts, USA). DNA purity and concentration were determined spectrophotometrically at 260 and 280 nm. The extracted DNA was stored at -20°C until analysis. Restriction fragment length polymerase chain

reaction (PCR-RFL) was performed to determine the different genotypes of IL6 - 174 G/C polymorphism. This polymorphism was analyzed by amplification of a 611-bp sequence using oligonucleotide primer sequences using the forward 5′-TGA CTT CAG CTT TAC TCT TGT-3′ and reverse 5′ -CTG ATT GGA AAC CTT ATT AAG-3′. The protocol consisting of an initial denaturation at 94°C for 4 min, followed by 30 cycles of annealing at 54 °C for 30 s, extension at 72 °C for 30 s and denaturation at 94 °C for 30 s, and a final extension at 72 °C for 4 min. The constituents of the reaction consisted of: 1.2 µM of each primer, 10mM of dNTPs, 2mM of MgCl2, 1 U of Taq DNA polymerase enzyme and 1× PCR buffer, along with 40–50 ng of DNA. All reactions were done using the thermal cycler Applied Biosystems 9600 (Per- kin Elmer, Singapore). The amplified fragment was digested with the restriction enzyme Nla III (NEW ENGLAND BioLabs^{W}) at 37 C for 4 hours and the products were then electrophoresed on 2% agarose gel, stained with ethidium bromide, and visualized by an ultraviolet transilluminator. DNA molecular weight marker (Qiagen Gel Pilot 100bp plus Ladder) (#Cat 239 045) was used to assess the size of the PCR-RFLP products. The amplified fragment (611bp) after digestion with Nla III restriction enzyme can either give rise to three fragments at 611, 367 and 244 bp, which indicates the presence of the heterozygous genotype (GC), or two fragments at 367 and 244 bp, which indicates the presence of the homozygous minor genotype (CC), or remains undigested as one fragment at 611 bp for the wild genotype (GG) [21].

2.3. Laboratory Assessment

Laboratory variables, including complete blood count (CBC), ESR, CRP, serum ferritin, IL-1beta, IL-6, S100A12 level were measured.

2.4. Statistical Analysis

Descriptive statistics concerning the relationship between the various clinical parameters and treatment response were calculated. The relationship between the different IL-6 polymorphisms and treatment response was assessed using the Chi-square test. P values were calculated via Mann-Whitney U test.

3. RESULTS

3.1. Patient Characteristics and Treatment Response

This cohort consisted of 60 s-JIA patients (37 males and 23 females) who were treated with TCZ during the course of their disease and were followed for a median of 5.6 years. Thirty-five patients received TCZ as first line therapy while 22 (patients had been treated with TNF inhibitors and then switched to TCZ. Table **1** shows the demographic and clinical characteristics of patients that did not differ according to the future response (or lack thereof) to TCZ.

At 6 months, 42 patients (70%) showed at least a transient clinical response and 15 (25%) had a poor clinical response. For 3 patients (5%), an assessment of the clinical response was not available (Figure **1**). We observed no significant differences in the distribution of the homozygous wild genotype (GG) (n = 22 [36.7%]), heterozygous (GC) (n = 32 [53.3%]), or homozygous minor genotype (CC) (n = 7 [11.7%]) across the different response groups (Table **2**). We then compared the frequencies of the different genotypes across different outcomes, including 1) clinical response, 2) subsequent switch or not to TNF blockades, 3) extent of improvement in the physician global assessment of disease, 4) achievement of a modified JADAS-10 score of 5 or better, 5) achievement of CID and 6) achievement of a glucocorticoid-free state (Table **2**). None of these outcomes was significantly associated with the different genotypes of IL6 -174.

Analysis of the data indicated according to the necessity to switch to a TNF inhibitor after TCZ showed that non-switchers had a lower duration from disease onset to diagnosis than switchers (median 0.07 years as compared to 0.29 years; P = 0.02).

3.2. Serum Biomarkers and Treatment Responses

Serum levels of S100A12 was different between the two response groups (Table **3**). These levels were analyzed and with regard to the transient clinical response or to switching to TNF blockades. We found that responders (TNF non-switchers) had higher serum S100A12 levels than non-responder (the TNF switchers). We have to mention that because most of our patients did not receive Tocilizumab at the time of blood sampling, we could not analyze serum level of IL-6 or IL-6 Ra. Patients with the highest and lowest serum levels of S100A12, IL 1 & CRP did not show significant correlation with the different genotypes of IL-6 [p= 0.072; OR=0.33; 95%CI (0.06-0.093), p=0.341 OR=0.63; 95%CI (0.08-0.043) and p=0.075; OR=0.39; 95%CI (0.07-0.079)], respectively.

Table 1: Demographic, Clinical and Laboratory Characteristics of the Patients with Systemic JIA at Baseline. Patients are shown all Together and Grouped according to the following Clinical Response Pattern at 6 Months

Variables	All patients (N=57)	Clinical response pattern at 6 months		p -value
		Transient/Good (N=42)	Poor (N=15)	
	N (%)	N (%)		
Male gender	37 (62)	25 (59)	10 (67)	0.67
Pattern of joint involvement None Arthralgia Oligoarthritis Polyarthritis	 7 (11.7) 14(23.3) 7(11.7) 35 (58.3)	 7(16.7) 10(23.8) 5(11.9) 19(45.2)	 0 (0.0) 1(6.7) 2(13.3) 13(86.7)	0.081
TNFi naïve at TCZ start	22 (38.6)	10 (23.8)	12 (80)	0.64
GC treatment at TCZ start	50 (83.3)	35 (83.3)	13 (86.7)	0.88
	Median (range)	**Median (range)**		
Age at symptom onset (years)	5.2 (0.5-17.3)	5.6 (0.5-17-4)	5.2 (0.6-13.0)	0.35
Age at disease diagnosis (years)	5.4 (0.5-17.5)	7.1(0.5-17.5)	5.3 (0.0-10.7)	0.48
Follow-up time (years)	5.6 (0.7-27.5)	4.4 (0.7-17.7)	8.6 (0.9-24)	0.08
Modified JADAS-10 score at TCZ start	17.0(5.0-33.0)	15.4(10.0-28.0)	20.0(13.0-30.0)	0.07
GC dose at TCZ start (mg/kg/d of PDN)	0.17 (0-15)	0.20(0-15)	0.18(0-10)	0.77

TNFi, tumor necrosis factor alpha inhibitor; TCZ, tocilizumab; GC, glucocorticoids; JADAS, juvenile arthritis disease activity score. p-value was calculated by Mann-Whitney U-test for continuous variables and by chi-square test for categorical variables. JADAS- is the sum of the physician global assessment of disease activity score (scale 0-10), count of joints with active arthritis(scale 0-10), and normalized C-reactive protein (CRP) level (scale 0-10;calculated as[CRP(in mg/liter)-10]/10, with a CRP level < 10mg/liter representing a score of zero).

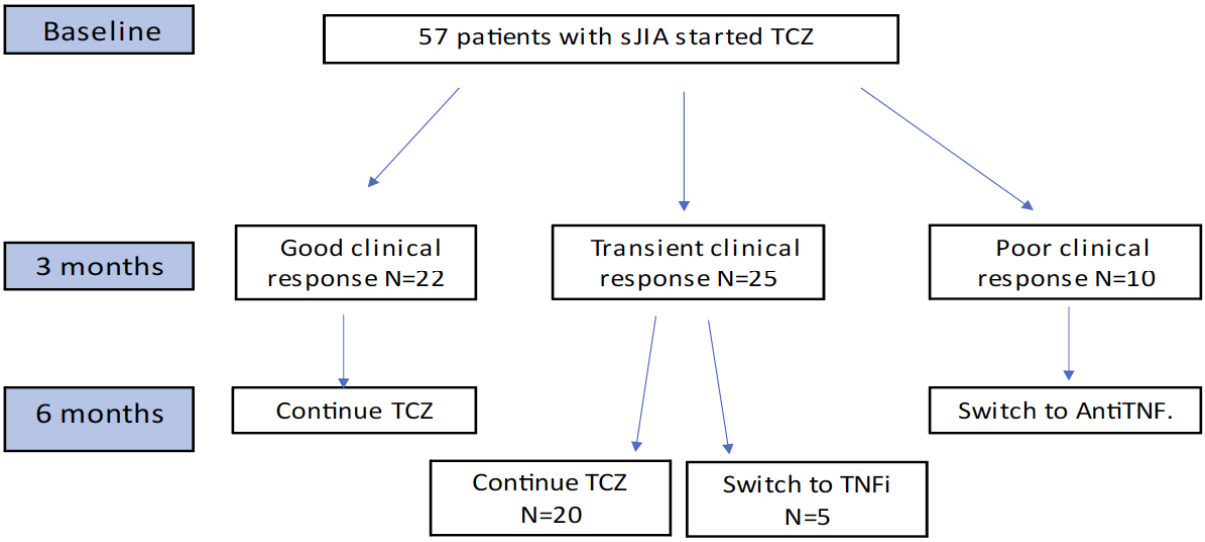

Figure 1: Flow Chart of 57 s-JIA patients included in this study.

4. DISCUSSION

Association of IL-6/IL-6R with s-JIA has made IL-6 as a central player in the pathology of this disease and led to the clinical trial of an IL-6 receptor (IL-6R) monoclonal antibody to block IL-6 classical and trans-signaling in an orphan disease such as s-JIA. In this cohort study of patients with systemic JIA who were treated with TCZ therapy, we could not find a significant association of IL-6 -174 G/C gene polymorphism with various definitions of an adequate treatment response in our cohort study. Association of IL-6 polymorphism and treatment response, however, was previously reported in French RA patients. In their study which included for 21 candidate genes, they found that the clinical response to treatment was

Table 2: Frequency of IL-6 Haplotypes in Patients with Systemic Juvenile Idiopathic Arthritis and their Relation to Treatment Responses at 6 Months

		Haplotype, N /total (%)			p value
	Patients with available data, N	Homozygous genotype (GG)	Heterozygous genotype (GC)	Homozygous minor genotype (CC)	
Clinical response					
At least transient	57	15/21	22/31	4/5	0.93
Good	57	13/21	20/31	4/5	
Any response *	59	5/22	7/30	5/7	
Best modified JADAS -10 score <5	35	5/11	7/20	3/4	0.76
CID	57	9/20	9/31	1/6	0.53
PGA score					
Improved at least 30%	50	11/16	26/30	2/4	0.83
Improved at least 50%	50	9/16	24/30	2/4	
Improved at least 70%	50	8/16	17/30	2/4	
Improved at least 90%	50	6/16	11/30	0/4	
GC therapy withdrawn	48	11/18	11/26	3/4	0.84
Not required switch to TNF inhibitors	60	13/22	22/32	6/7	0.41

*Defined as improvement in fever (if present) and/or arthritis (if present). JADAS-10, Juvenile Arthritis Disease Activity Score in 10 joints; CID, clinically inactive disease; GC, glucocorticoids. PGA, physician global assessment of disease activity.

Table 3: Biomarker Levels in Responders vs. Non-Responders to TCZ Therapy

Serum Biomarkers	Clinical response			Response based on treatment pattern		
	No response	Good/ transient response	P value	Subsequent switch to Anti TNF	No Subsequent switch to Anti TNF	P value
IL-6, pg/ml	5.43 (0.77-86.46)	6.92 (1.52-133.19)	0.33	5.23 (0.78-86.45)	6.83 (1.44-142.29)	0.26
S100A12, ng/ml	35.7 (3.4-261.4)	48.3 (4.0-751.7)	0.71	34.7 (3.4-261.4)	49.1 (4.0-731.6)	0.72
IL-1beta, pg/ml	9.43 (2.63-18.25)	9.43 (3.37-75.31)	0.94	9.42 (2.63-18.45)	9.42 (3.38-75.33)	0.91
CRP, mg/dl	46 (24-87)	54.85(14.5-112)	0.399	43(24-80)	55.35(14.5-112)	0.381
Ferritin, ng/ml	156(44-570)	167.5 (100-700)	0.067	155(45-560)	167.5 (100-700)	0.087

TCZ= Tocilizumab, data is expressed as median (interquartile range), P values were calculated with the Mann-Whitney U test.

associated with SNP genotype in the gene IL6R, where patients with the homozygous AA-genotype for rs12083537 (IL6R) showed significant better response than homozygous or heterozygous patients with the G allele [22]. It has also been found in RA patients that AA genotype for rs12083537 and CC for rs11265618 polymorphisms may act as predictors of good response to TCZ, where the patients treated with TCZ showed better EULAR response, remission, low disease activity (LDA) and DAS28 improvement rates [23].

Interestingly, whereas an association of the different IL6R SNPs with the risk of systemic JIA was seen in several of the systemic JIA cohorts (from the US, UK, Italy, Brazil, Canada, Spain and Argentina) reported by Arthur *et al.* [18]. Therefore, it is possible that genetic risk factors for s-JIA in particular for non-responders vary in different populations. Among the response group in our study, the homozygous minor genotype (CC) (n = 7 [11.7%]) showed the lower frequencies. In fact, this finding had come in agreement with Ogilvie *et al.* [24] who reported significantly reduced frequency of the -174 CC genotypes in systemic JIA patients with age at onset of ≤5 years. Therefore, the reduction in the frequency of the CC genotype in JIA patients suggested that this genotype confers a protective influence against the development of the disease. As well as IL-6 response to a stressful stimulus [24]. On the other hand, Ciccarelli *et al.* [25] found that IL-6 gene (-174 G/C) has been associated with bone

erosive damage in RA patients especially those with CC genotype. The low frequency of the C allele in Indians and the reduction in CC genotype frequency in children aged ≤5 years are very interesting [26, 27] and actually similar to our findings. In addition, IL-6 plays a central role in contributing to the development of the disease in which it is considered as a pro-inflammatory cytokine that had different pleiotropic activities including induction of an acute phase proteins and stimulation of T as well as B cells. These reactions result in cartilage and bone damage as well as other systemic manifestations [28]. A polymorphism in -174G/ C of the IL-6 gene promoter region was reportedly associated with systemic onset juvenile idiopathic arthritis and susceptibility to RA in Europeans [18]. In the cohort study by Arthur or Claas Hinze *et al.,* the patients are not well characterized, making its comparisons with our cohort less feasible. Arthur *et al.* [18], included 38 patients in their study, and demonstrated that high expression alleles of systemic JIA–associated IL1RN SNPs were strongly associated with non-responsiveness to anakinra therapy. The lack of association between these SNPs and non-responsiveness to Tocilizumab treatment suggests that these SNPs are specifically associated with anakinra non-responsiveness, as opposed to being associated with more global treatment resistance. However, Claas Hinze *et al.* [29] included larger population size of 61 patients and could not confirm an association of IL1RN haplotypes and SNPs with response to IL-1 blockade in their cohort of patients with systemic JIA. Also, patients who received Tocilizumab following IL-1 blockade had a longer duration from disease onset to diagnosis than those who did not receive tocilizumab. Regardless this controversy in the genetic association of IL-6 and s-JIA, the IL-6 blockade (TCZ) has proven to have remarkable efficacy in this disease in a proof-of-principle phase I/II study with a single dose and in a separate dose-escalation study [30, 31]. Moreover, the results of our cohort study indicate that non-responder JIA patients, showed a longer duration from the onset of symptoms to diagnosis (median 0.29 years) when compared to those with responders (median 0.07 years). Hence, this observation supports the “window of opportunity” hypothesis, i.e., early diagnosis and treatment may positively influence the long-term outcome of the disease [32]. However, we did not find a significant association between the duration from the onset to diagnosis with different measures of clinical responses to treatment. Furthermore, we could not find a significant correlation between serum IL-1beta, CRP, S100A12 and IL6 -174 different genotypes; therefore, based on our current data, it would not be possible to conclude on the effect of IL6 -174 different genotypes on severity of inflammation or response to TCZ therapy. However, recent data indicate a prominent role of IL-1 in s-JIA. Sera of s-JIA patients induce the transcription of genes of the innate immune system including IL-1 in peripheral blood mononuclear cells (PBMCs). In addition, activated monocytes from patients with s-JIA secrete significantly higher amounts of IL-1 in comparison with monocytes of healthy controls, whereas release of IL-6 was not significantly different in both groups [33]. In addition, IL-1 acts on the bone marrow and stimulates granulopoiesis, activates the thermoregulation of the hypothalamus and leads to fever. Furthermore, IL-1 receptor activation on endothelial cells that may cause cutaneous rash in s-JIA and result in the production of IL-6 [34]. IL-6 on the other hand, stimulates hepatocytes and induces the production of several acute-phase proteins like CRP and serum amyloid A. Serum levels of IL-6 are markedly elevated in patients with S-JIA and correlated with systemic features of the disease, especially with periods of fever and platelet counts as well as severity of joint involvement [35]. On contrary to our cohort, genetic studies on a functional relevant single-nucleotide polymorphism of the IL-6 promoter confirm the role of IL-6 in S-JIA. Differences in transcriptional levels driven by the -174 G/C alleles in the 50-flanking region of the IL-6 promoter suggest that the polymorphism in the IL-6 gene itself contribute to the overproduction of IL-6. Controls with -174 CC genotype had lower serum levels of IL-6 than those with GG genotype. There was a significant under-representation of the -174 CC genotype in patients with S-JIA compared with controls [36,37]. Moreover, Artsymovych *et al.* found that, certain phenotypes of the JIA may be distinguished depending on the allele polymorphism of the IL-6 gene. Thus, revealing the polymorphism of these alleles in patients at the onset of the disease, may help to predict to some extent its course and take this into account when choosing treatment tactics.[38] In this regard, S100A12 seems to be a member of a novel inflammatory signaling pathway involving RAGE (receptor for advanced glycation end products) as a receptor transducing pro-inflammatory signals in endothelial cells and phagocytes [39,40]. It induces the expression of adhesion molecules as well as other pro-inflammatory cytokines on endothelial cells. Direct effects of S100A12 on expression of IL-1 and IL-6 have not been described so far [41].

We acknowledge that our study displays some limitations. First, we evaluated a cohort of patients with variable duration of follow-up, which may have an impact on the treatment outcomes; for example, a patient with a much longer disease course may have a higher chance to receive TNF blockers following TCZ. In addition we were unable to extract formal response criteria from the registry, such as the modified ACR Pedi 30 response criteria or the complete JADAS criteria, this was because some of the criteria, such as patient global assessment of disease activity, were not recorded. Nevertheless, from a clinical standpoint, we believe that our assessment of treatment response, i.e., clinical response concerning systemic inflammation and drug survival, was valid. Because patients were often not enrolled in the registry early during the disease, the laboratory data recorded in the registry almost certainly do not represent the most prominent changes, since such data are often present at the onset of disease; therefore, the effect of certain laboratory abnormalities on treatment outcomes are very limited. Finally, the assessment of serum cytokine levels, including IL-6Ra, is presumably affected by multiple factors, such as degree of systemic inflammation and current medications, for which we did not control. Therefore, based on our current data, it would not be possible to conclude on the effect of IL6 -174 different genotypes on IL-6 Ra expression. It would be desirable to extend genetic studies to cohorts that could be better characterized and prospectively followed up.

5. CONCLUSION

We found a non-significant impact of IL6 -174 G/C gene polymorphisms on treatment response to IL6 blockade therapy (Tocilizumab). However, this study provides evidence of a “window of opportunity”; improved long-term treatment response with shorter time from disease onset to diagnosis and hence treatment, presumably.

ACKNOWLEDGEMENT

We appreciate the role of research square in which a preprint of this manuscript has previously been published [42].

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

All Authors contributed to the manuscript and approved the final version.

ETHICS APPROVAL

The protocol of the present study was registered by the local ethics committee of Tanta University Hospital with approval code 34571/5/20.

PATIENT CONSENT FOR PUBLICATION

Written informed consents were collected from patients’ parents.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

DATA AVAILABILITY STATEMENT

All data relevant to the study are included in the article.

REFERENCES

[1] Symmons D, Jones M, Osborne J, Sills J, Southwood T, Woo P. Pediatric rheumatology in the United Kingdom: data from the British Pediatric Rheumatology Group National Diagnostic Register. J Rheum1996; 11: 13-16.

[2] Laxer R, Schneider R. Systemic-onset juvenile chronic rheumatoid arthritis and adult-onset Still’s disease. In Oxford Textbook of Rheumatology. Maddison P, Isenberg D, Woo P, Glass D, Eds. Oxford Medical Publications, Oxford 1993; 722-731.

[3] Wallace CA, Levinson JE. Juvenile rheumatoid arthritis: outcome and treatment for the 1990s. Rheum Dis Clin North Am 1991; 17: 891-905. https://doi.org/10.1016/S0889-857X(21)00134-4

[4] Fishman D, Rooney M, Woo P. Successful management of reactive haemophagocytic syndrome in systemic-onset juvenile chronic arthritis. Br J Rheumatol 1995; 34: 888. https://doi.org/10.1093/rheumatology/34.9.888

[5] De Benedetti F, Massa M, Robbioni P, Ravelli A, Burgio GR, Martini A. Correlation of serum interleukin-6 levels with joint involvement and thrombocytosis in systemic juvenile rheumatoid arthritis. Arthritis Rheum 1991; 34: 1158-1163.

[6] Lepore L, Pennesi M, Saletta S, Perticarari S, Presani G, Prodan M. Study of IL-2, IL-6, TNF alpha, IFN gamma and beta in the serum and synovial fluid of patients with juvenile chronic arthritis. Clin Exp Rheumatol 1994; 12: 561-565. https://doi.org/10.1002/art.1780340912

[7] Mangge H, Kenzian H, Gallistl S, Neuwirth G, Liebmann P, Kaulfersch W, Beaufort F, Muntean W, Schauenstein K. Serum cytokines in juvenile rheumatoid arthritis. Correlation with conventional inflammation parameters and clinical subtypes. Arthritis Rheum 1995; 38: 211-220. https://doi.org/10.1002/art.1780380209

[8] De Benedetti F, Pignatti P, Massa M, Sartirana P, Ravelli A, Martini A. Circulating levels of interleukin 1 beta and of

interleukin 1 receptor antagonist in systemic juvenile chronic arthritis. Clin Exp Rheumatol 1995; 13: 779-784.

[9] Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nat Immunol 2015; 16: 448-57. https://doi.org/10.1038/ni.3153

[10] McInnes IB, Buckley CD, Isaacs JD. Cytokines in rheuatoid arthritis - shaping the immunological landscape. Nat Rev Rheumatol 2016; 12: 63-8. https://doi.org/10.1038/nrrheum.2015.171

[11] Gibofsky A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. Am J Manag Care 2012; 18(13 Suppl): S295-302.

[12] Heinrich PC, Behrmann I, Haan S, *et al*. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 2003; 374(Pt 1): 1-20. https://doi.org/10.1042/bj20030407

[13] Jones SA, Scheller J, Rose-John S. Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. J Clin Invest 2011; 121: 3375-83. https://doi.org/10.1172/JCI57158

[14] Yokota S, Miyamae T, Imagawa T, Iwata N, Katakura S, Mori M, Woo P, Nishimoto N, Yoshizaki K, Kishimoto T. Therapeutic efficacy of humanized recombinant anti-interleukin-6 receptor antibody in children with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 2005; 52: 818-825. https://doi.org/10.1002/art.20944

[15] Wolford JK, Bcolligan P, Gruber JD, Bogardus C. Variants in the interleukin 6 receptor gene are associated with obesity in Pima Indians Metabolism 2011; 60(10): 1488-1496.

[16] Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, *et al*. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton. J Rheumatol 2001; 31: 390-2.

[17] Bielak M, Husmann E, Weyandt N, Haas JP, Hügle B, Horneff G, *et al*. IL-6 blockade in systemic juvenile idiopathic arthritis— achievement of inactive disease and remission (data from the German AID-registry). Pediatr Rheumatol Online J 2018; 16: 22. https://doi.org/10.1186/s12969-018-0236-y

[18] Arthur VL, Shuldiner E, Remmers EF, Hinks A, Grom AA, Foell D, *et al*. IL1RN variation influences both disease susceptibility and response to recombinant human interleukin-1 receptor antagonist therapy in systemic juvenile idiopathic arthritis. Arthritis Rheumatol 2018; 70: 1319-30. https://doi.org/10.1002/art.40498

[19] Wallace CA, Giannini EH, Huang B, Itert L, Ruperto N, for the Childhood Arthritis and Rheumatology Research Alliance (CARRA), Pediatric Rheumatology Collaborative Study Group (PRCSG), Paediatric Rheumatology International Trials Organisation (PRINTO). American College of Rheumatology provisional criteria for defining clinical inactive disease in select categories of juvenile idiopathic arthritis. Arthritis Care Res (Hoboken) 2011; 63: 929-36. https://doi.org/10.1002/acr.20497

[20] Consolaro A, Ruperto N, Bazso A, Pistorio A, Magni-Manzoni S, Filocamo G, *et al*. for the Paediatric Rheumatology International Trials Organisation. Development and validation of a composite disease activity score for juvenile idiopathic arthritis. Arthritis Rheum 2009; 61: 658-66. https://doi.org/10.1002/art.24516

[21] Revillaa M, Obacha V, Cerveraa A, Davalosb A, Castilloc J, Chamorroa A. A -174G/C polymorphism of the interleukin-6 gene in patients with lacunar infarction. J Neuroscience Letters 2002; 324: 29-32. https://doi.org/10.1016/S0304-3940(02)00169-6

[22] Luxembourger C, Ruyssen-Witrand A, Ladhari C, Rittore C, Degboe Y, *et al*. A single nucleotide polymorphism of IL6-receptor is associated with response to tocilizumab in rheumatoid arthritis patients. Pharmacogenomics J 2019; 19(4): 368-374. https://doi.org/10.1038/s41397-019-0072-6

[23] Maldonado-Montoro M, Cañadas-Garre M, González-Utrilla A, Ángel Calleja-Hernández Influence of IL6R gene polymorphisms in the effectiveness to treatment with tocilizumab in rheumatoid arthritis. Pharmacogenomics J 2018; 18(1): 167-172. https://doi.org/10.1038/tpj.2016.88

[24] Ogilvie EM, Fife MS, Thompson SD, Twine N, Tsoras M, Moroldo M, Fisher SA. The -174G Allele of the Interleukine-6 Gene Confers Susceptibility to Systemic Arthritis in Children. A Multicenter Study Using Simplex and Multiplex Juvenile Idiopathic Arthritis Families. Arthritis and Rheumatism 2003; 48(11): 3202-3206. https://doi.org/10.1002/art.11300

[25] Ceccarelli F, Perricone C, Fabris M. Transforming growth factor β 869 C /T and interleukin-6 -174G/C polymorphisms relate to the severity and progression of bone-erosive damage detected by ultrasound in rheumatoid arthritis. Arthritis Res Ther 2011; 13(4): R1-11. https://doi.org/10.1186/ar3396

[26] Murray K, Donnelly P, Passo M, Giannini E, Glass D. Windows of effect of disease associated HLA alleles in juvenile rheumatoid arthritis. Arthritis Rheum 1997; 40: S2-41.

[27] Al-Harbi SJ, Hadi MA, Rashid KH. Association of IL-6 gene−174 G/C (rs1800795) Polymorphism with Juvenile Idiopathic Arthritis in Iraqi Patients. Indian Journal of Forensic Medicine & Toxicology, October-December 2019; 13(4). https://doi.org/10.5958/0973-9130.2019.00397.9

[28] Kishimoto T. Interleukin-6: from basic science to medicine: 40 years in immunology. Annu Rev Immunol 2005; 23: 1-21. https://doi.org/10.1146/annurev.immunol.23.021704.115806

[29] Hinze C, Fuehner S, Kessel C, Wittkowski H, Lainka E, *et al*. Impact of IL1RN Variants on Response to Interleukin-1Blocking Therapy in Systemic Juvenile Idiopathic Arthritis. Arthritis & Rheumatology 2020; 72(3): 499-505. https://doi.org/10.1002/art.41130

[30] Yokota S, *et al*. Therapeutic efficacy of humanized recombinant anti-interleukin-6 receptor antibody in children with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 2005; 52(3): 818-825. https://doi.org/10.1002/art.20944

[31] Woo P, *et al*. Open label phase II trial of single, ascending doses of MRA in Caucasian children with severe systemic juvenile idiopathic arthritis: proof of principle of the efficacy of IL-6 receptor blockade in this type of arthritis and demonstration of prolonged clinical improvement. Arthritis Res Ther 2005; 7(6): R1281-R1288.

[32] Nigrovic PA. Is there a window of opportunity for treatment of systemic juvenile idiopathic arthritis? [review]. Arthritis Rheumatol 2014; 66: 1405-13. https://doi.org/10.1002/art.38615

[33] Pascual V, Allantaz F, Arce E, Punaro M, Banchereaux J. Role of interleukin (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med 2005; 201: 1479-86. https://doi.org/10.1084/jem.20050473

[34] Dinarello CA. Blocking IL-1 in systemic inflammation. J Exp Med 2005; 201: 1355-9. https://doi.org/10.1084/jem.20050640

[35] De Benedetti F, Massa M, Robbioni P, Ravelli A, Burgio GR, Martini A. Correlation of serum interleukin-6 levels with joint involvement and thrombocytosis in systemic juvenile rheumatoid arthritis. Arthritis Rheum 1991; 34: 1158-63. https://doi.org/10.1002/art.1780340912

[36] Fishman D, Faulds G, Jeffery R, *et al*. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6

transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998; 102: 1369-76. https://doi.org/10.1172/JCI2629

[37] Ogilvie EM, Fife MS, Thompson SD, *et al*. The -174G allele of the interleukin-6 gene confers susceptibility to systemic arthritis in children. Arthritis Rheum 2003; 48: 3202-6. https://doi.org/10.1002/art.11300

[38] Artsymovych A, Oshlianska O, Rossokha Z. Allelic polymorphism of proinflammatory cytokine genes as a basis for the formation of phenotypes of juvenile idiopathic arthritis. Abstract in; Basic and translat. scien. in paediat. Rheum.AB0173.Annals of rheum.dis, 1136, 2020 eular 2300, 79(suppl.1). https://doi.org/10.1136/annrheumdis-2020-eular.2300

[39] Hofmann MA, Drury S, Fu C, *et al*. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. Cell 1999; 97: 889-901. https://doi.org/10.1016/S0092-8674(00)80801-6

[40] Foell D, Wittkowski H, Vogl T, Roth J. S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J Leukoc Biol 2007; 81: 28-37. https://doi.org/10.1189/jlb.0306170

[41] Newton RA, Hogg N. The human S100 protein MRP-14 is a novel activator of the beta(2) integrin Mac-1 on neutrophils. J Immunol 1998; 160: 1427-35.

[42] https://www.researchsquare.com/article/rs-725673/v1.

Received on 25-03-2022 Accepted on 30-05-2022 Published on 07-06-2022

https://doi.org/10.12970/2310-9874.2022.10.01